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Assessment of Growth, Metallic Ion Accumulation, and Translocation of Lavandin *(Lavandula × intermedia)* Plant in Cadmium Stress

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ABSTRACT

Excess cadmium (Cd), which is toxic to plants, severely limits crop production in agricultural areas. For this reason, this study investigated the effect of increased Cd levels on lavandin growth, some physiological parameters, and metallic ion accumulation and translocation. In greenhouse conditions, six different levels of Cd (0, 25, 50, 100, 150, and 200 µM Cd) were applied to plants grown in perlite medium together with a complete nutrient solution. Increasing Cd levels decreased biomass production in both the shoots and roots and the contents of chlorophyll (Chl) *a, b, a+b*, and carotenoid (Car). In addition, excessive Cd decreased the concentrations of some metallic cations such as iron (Fe), zinc (Zn), manganese (Mn), and calcium (Ca) in the shoots and roots. Similarly, increasing Cd decreased the bio-concentration factor (BCF) of the metallic cations (BCF of Cd, Fe, Mn, and Zn in both the shoots and roots and the BCF of copper (Cu) in the roots. Toxic Cd levels decreased the translocation factor (TF) of Zn and Cu and the net accumulation (NA) via roots in Fe and Zn. The effect of Cd on the NA via roots in K, Ca, Mn, and Cu was not found to be significant. However, increasing Cd caused an increase in shoot and root membrane permeability and the TF of Fe and Mn. It was concluded that Cd2+ ion interacts divalent cations such as Ca2+, Fe2+, Zn2+, and Mn2+ ions and could affect the concentrations of these ions in the shoots and roots, and excess Cd has a negative effect on the growth and the photosynthetic capacity of lavandin.

Keywords: Bio-concentration, Chlorophyll, Membrane permeability, Metal translocation, Nutrient imbalance, *Lavandula hybrida*

1. Introduction

Cadmium (Cd), a non-nutritive element for plants, is regarded as toxic to plants and other living organisms and has serious pollutant effect on the environment. This metal's contamination of agricultural soils is largely driven by mining activities such as the smelting of Zn-containing ores, burning of fossil fuels, incineration of wastes, use of sewage sludge as fertilizer, contaminated irrigation water, and the applications of phosphorus fertilizers (Alloway & Steinnes 1999). The fact that it is an easily soluble element in water increases its polluting capacity and toxicity effects on the environment.

In plants, Cd toxicity causes the most visible symptoms through chlorosis, leaf curls, and stunting (Benavides et al. 2005), while it also inhibits plant growth (Hediji et al. 2021), and decreases photosynthetic activity (Sandalio et al. 2001; Irshad et al. 2021). In addition, Ekmekçi et al. (2008) reported that excess Cd can cause an increase in reactive oxygen species at the cellular level and thus damages membranes, cell molecules, and organelles. In addition, it may cause a decrease in nutrient and water uptake and ion translocation, an increase in oxidative damage, and disruptions in a plant's metabolism (Haider et al. 2021). Cd toxicity may also adversely affect the uptake and transfer of K, P, Ca, Mg, and Mn (Nazar et al. 2012). Previous studies in relation to Cd toxicity have provided significant findings concerning plant growth in pea plants (Sandalio et al. 2001), in Indian mustard (Goswami & Das 2015), and beans (Hediji et al. 2021). Excess Cd also induces leaf chlorosis accompanied by a lowering of the photosynthetic rate in maize cultivars (Ekmekçi et al.

2008) and wheat (Irshad et al. 2021). Angelova (2012) reported that some medicinal and aromatic plants, such as *Valeriana officinalis* L. and *Melissa officinalis* L., uptake heavy metals from the root zone through the root system and accumulate a large part of them in their roots. In a later study Angelova et al. (2015) states that the Lavandin plant, which is more tolerant to heavy metals, can grow in polluted soils and generally accumulate heavy metals in various organs, with the exception of essential oils.

The genus *Lavandula* belongs to the *Lamiaceae* family and includes cultivars and hybrids of about 40 species. Lavandin, which is called hybrid lavender/or hardy lavender, is widely grown in the Mediterranean basin, especially on calcareous soils, and appears as a shrub form that can grow up to 70-80 cm. The interest in this species has increased in recent years due to its high yield of essential oils, its ability to grow in contaminated or barren agricultural lands, and being wide in demand for its oil in the food and cleaning industry (Platt 2009). In addition, its appealing appearance during the flowering period makes it popular for agro-tourism and urban parks (Veeck et al. 2016). This study determines the effects of Cd on plant growth and concentration, accumulation, and translocation of ions in lavandin plants. In addition, this study not only gives an idea about the behaviour of Cd in a plant belonging to the *Lamiaceae* family but also presents the potential for the plant to be grown in Cd-contaminated soils as aromatic as well as ornamental plants.

2. Material and Method

2.1. The experimental design

The greenhouse experiment was carried out with lavandin (*Lavandula × intermedia*), a hybrid of *L. angustifolia* and *L. latifolia*, in the summer season of 2017 and lasted for 30 days. The average air temperature and relative humidity were measured at 26/17 °C (day/night) and 65%, respectively. Four-month-old seedlings were obtained from a private company producing outdoor ornamental plants and transferred to 2 L capacity polyethylene pots (one plant per pot) in the hydroponic system [modified Hoagland solution (Hoagland & Arnon 1950) and perlite as an inert media] inside a greenhouse under natural light conditions. Prior to Cd application, the lavandin saplings were irrigated with different rates of modified Hoagland solution for 12 days (four days quarter-strength, four days half-strength, and four days full-strength) for acclimatization. This solution contained calcium nitrate tetrahydrate [$Ca(NO_3)_2.4H_2O$] (5 mM), potassium nitrate (KNO_3) (5 mM), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) (2 mM), potassium di-hydrogen phosphate (KH_2PO_4) (1 mM), boric acid (H_3BO_3) (45.5 µM), iron sulphate heptahydrate ($FeSO_4.7H_2O$) (44.7 µM), sodium chloride (NaCl) (30.0 µM), manganese sulphate monohydrate ($MnSO_4.H_2O$) (9.1 µM), zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) (0.77 µM), copper sulphate pentahydrate ($CuSO_4.5H_2O$) (0.32 µM), ammonium molybdate tetrahydrate [$(NH_4)_2Mo_7O_{24}.4H_2O$] (0.10 µM), and disodium EDTA dihydrate ($Na_2EDTA.2H_2O$) (54.8 µM). The pH of the nutrient solution was maintained at 6.5 throughout the experiment.

The saplings were exposed to six applications; including control (0), 25, 50, 100, 150, and 200 µM Cd (as $CdCl_2$). These levels were dissolved separately in a full-strength modified Hoagland solution in different containers. During the period of the experiment, the plants were irrigated with these solutions (100 mL per day). The Cd levels were selected based on the doses used in previous studies and the toxicity of these doses to various plant species (Cikili et al. 2016; Kaya et al. 2009).

2.2. Procedures in sampling and harvesting

At the end of the 30th day, the harvested plants were separated into shoots and roots to determine the weights of biomass (the FWs and DWs). The aerial parts were weighed for shoot FW. The roots were carefully cleaned from the soil and dipped for 15 minutes in an aerated 0.5 mM calcium chloride ($CaCl_2$) solution to remove the nutrients adsorbed from the root surface and weighted for root FW. In order to remove any particles that may have adhered to plant surfaces, samples were washed three times under running tap water and rinsed with deionized water. Afterwards, all shoots and roots were kept in an oven with regulated ventilation at 70 °C in order to dry them until their weight was stable. The shoot and root dry weights of the dried plant samples were determined separately and then ground for nutritional ion analysis.

2.3. Determination of photosynthetic pigments and membrane permeability

The membrane permeability (MP) in the disk samples taken from fresh leaves was measured by electrical conductivity (EC%) (Yan et al. 1996). Briefly, 1 cm^2 pieces of washed fresh leaf were placed in a beaker containing 10 mL (30 °C) deionized water for three hours, and the conductivity of the solution was then measured (EC_1). After boiling the same samples for 20 mins, they were cooled to room temperature and their conductivity was measured again (EC_2). The MP (EC %) was calculated using the formula [$(EC_1/EC_2) \times 100$].

The photosynthetic pigment contents were measured in fully expanded young leaves immediately before harvest. For this, fresh leaf samples (0.25 g) were cut into small pieces and homogenized using a homogenizer (Heidolph DIAX 900, Kelheim, Germany) in 10 mL of acetone (90%, v/v).

The absorbance of the filtered extract was determined at 663, 645, and 470 nm using a spectrophotometer (Shimadzu UV-1201, Japan), and then the Chl *a*, Chl *b*, and carotenoid (Car) contents were calculated, respectively, according to the formula reported by Lichtenthaler (1987).

2.4. Determination of metal ions

To measure the nutrient ion concentrations, 500 mg of each of the shoot samples were dry-ashed in a muffle furnace at 500 °C for 6 h, and then the cooled ash was dissolved in 10 N nitric acid (HNO_3) solution (Miller 1998). The concentrations of Cd, Fe, Zn, Mn, and Cu were determined using ICP-OES (Perkin Elmer Optima 2100 DV; Waltham, MA). The concentration of K and Ca were analysed using a flame photometer.

2.5. Determination of bio-concentration, translocation, and accumulation

The capacity of the lavandin plant to accumulate Cd was evaluated based on the bio-concentration factor (BCF), the translocation factor (TF), the total accumulation rate (TAR), and net ion accumulation (NA) via roots. The BCF is defined as the ratio of the total metal concentration in the aerial parts to the metal concentration in the rooting media, and it is accepted as an indicator of the ability to absorb metals and transport them to the shoots (Cikili et al. 2016). The TF value is defined as the ratio of heavy metal concentration in the shoot to that in the root (Cikili et al. 2016). The TAR, which is a useful parameter for bioaccumulation studies, is a measure of plants' heavy metal uptake (Zhu et al. 1999). The NA of ions via roots is the rate of total ion quantities in the whole plant to root DW. The last two values have been calculated using equations [1] and [2], respectively (Moradi & Ehsanzadeh 2015).

$$\text{TAR of Cd (µg/g DW/day)} = (Cd_{shoot} \times DW_{shoot}) + (Cd_{root} \times DW_{root}) / \text{growth day} \times (DW_{shoot} + DW_{root}) \quad [1]$$

$$\text{NA of ions via roots (mg or µg / g DW)} = [ion]_{shoot} / DW_{root} \quad [2]$$

where $[ion]_{shoot}$ or $_{root}$ is ion concentration in shoot or root.

2.6. Statistical analysis

The experiment was designed in a completely randomized factorial design (in three replications) and the data obtained were analysed using ANOVA with the MINITAB package program (Minitab Corp., State College, PA). The differences between applications were analysed using Duncan's multiple range test at the significance level (α=0.05).

3. Results

3.1. Shoot and root biomass

A significant decrease in shoot and root FWs and DWs of lavandin were found with increasing Cd levels (Figure 1). These decreases varied from 25.9% and 39.1% in shoot FW, 31.3% and 50.7% in root FW, 26.4% and 49.6% in shoot DW, and 33.8% and 44.1% in root DW.

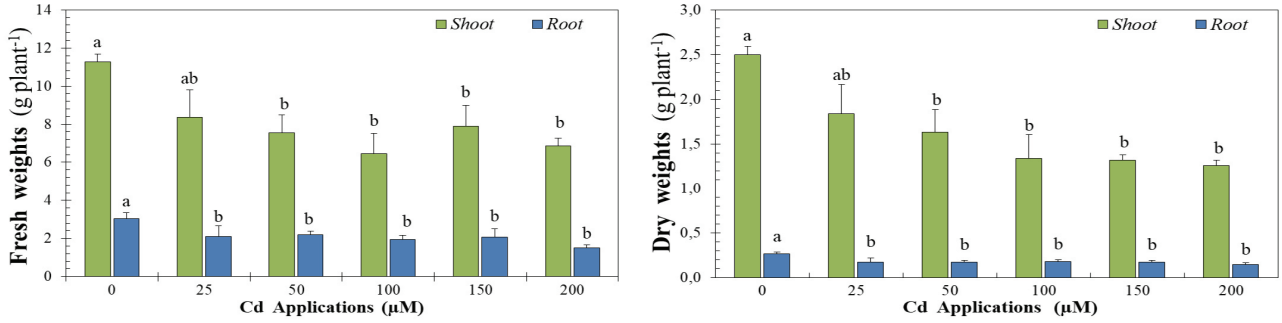

Figure 1- The effect of Cd toxicity on fresh and dry weights of leaves and roots of lavandin. (Bars indicate means of three replicates ± SE. Different letters on the bars for each parameter differ significantly according to DMRT)

3.2. Membrane permeability and photosynthetic pigment contents

The increase in Cd levels considerably affected the MP values and photosynthetic pigment contents of the lavandin leaves compared to the control (Figure 2). Particularly high Cd levels (150 and 200 µM) resulted in a significant increase in the MP values in the roots of lavandin at 29.5% and 31.6% and also in its leaves at 26.3% and 28.0%, respectively. On the other hand, increasing Cd levels (25, 50, 100, 150, and 200 µM) caused a notable decrease in Chl *a*, Chl *b*, Chl *a*+*b*, and Car content in the lavandin leaves. These decreases in Chl *a* content were found to be 13.5%, 24.6%, 43.8%, 64.8, and 57.8%, respectively. Similar decreases in Chl *b*, Chl *a*+*b*, and Car contents of lavandin were also found (Figure 2).

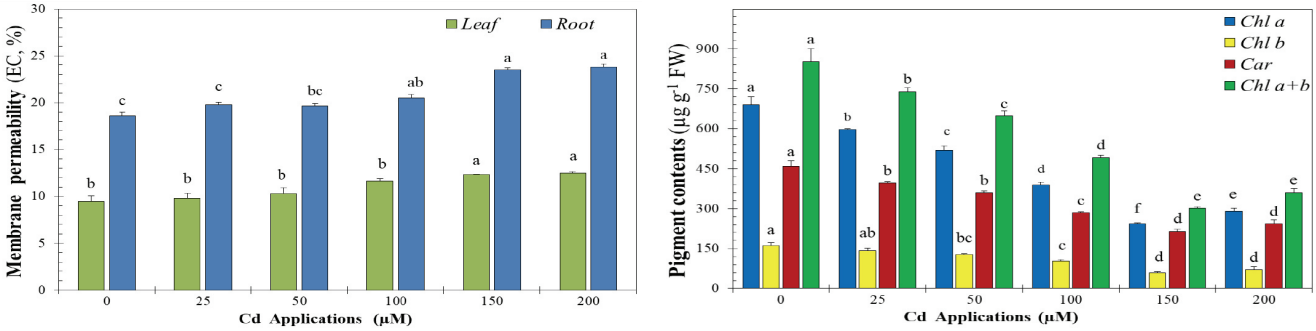

Figure 2- The effect of Cd toxicity on membrane permeability in leaves and roots, and photosynthetic pigment contents in leaves of lavandin (Bars indicate means of three replicates ± SE. Different letters on the bars for each parameter differ significantly according to DMRT)

3.3. Concentration, translocation and accumulation of metallic nutrients

Shoot and root Cd concentrations increased considerably with all Cd applications. Those increases with the lowest and highest Cd levels ranged from 7.3-fold to 27.5-fold in the shoots and from 39.8-fold to 96.0-fold in the roots (Table 1). At the same time, the shoot and root BCF of lavandin plant showed a decreasing tendency depending on increasing Cd levels (Table 1). While the root BCF value of Cd was found by 1.56 at the lowest Cd (25 µM) level, it was 0.73 at the highest Cd (200 µM) level. Similarly, these decreases in the roots ranged from 293 to 88 in the lowest and highest level of Cd. The TF of Cd significantly decreased with all Cd levels compared to the control. All Cd levels (25, 50, 100, 150, and 200 µM Cd) caused a marked increase in the TAR of Cd at 30.8-, 54.1-, 92.9-, 95.6-, and 92.4-fold, respectively.

Table 1-The effect of Cd toxicity on concentrations, bio-concentration, translocation, and accumulation of Cd in lavandin

Treated Cd (µM)	*Concentrations of Cd (µg g^{-1} DW)*		*BCF of Cd*		*TF of Cd*	*TAR of Cd (µg g^{-1} DW day^{-1})*
	Shoot	*Root*	*Shoot*	*Root*		
0	0.6±0.03^{e}	20.7±1.59^{e}	-	-	2.71±0.37^{a}	0.08±0.01^{c}
25	4.4±0.20^{d}	824.1±7.36^{d}	1.56±0.07	293±2.6	0.53±0.02^{b}	2.46±0.14^{b}
50	5.1±0.21^{d}	1278.1±40.4^{c}	0.90±0.04	227±7.2	0.40±0.02^{b}	4.33±0.29^{b}
100	10.0±0.39^{c}	1756.7±156^{b}	0.89±0.04	156±13.9	0.58±0.07^{b}	7.43±1.38^{a}
150	14.2±1.13^{b}	1847.5±51.0^{ab}	0.84±0.07	110±3.0	0.77±0.07^{b}	7.65±0.87^{a}
200	16.5±0.54^{a}	1988.0±50.6^{a}	0.73±0.02	88±2.3	0.83±0.04^{b}	7.39±0.49^{a}
F-test	***	***	-	-	***	***

All values are the average of three replicates (means ± SE, n=3). Different letters in the same column differ significantly according to the DMRT. *F*-test shows significant difference at ***, p<0.001

The effects of Cd levels on the K concentrations in the shoot and root were not found to be significant in the lavandin plant, compared to the control (Figure 3). However, Cd levels caused a notable decrease in Ca concentration in the shoots and roots. These decreases ranged from 15.8% to 28.6% in the shoots and from 24.5% to 44.8% in the roots.

Increasing Cd levels were also found to significantly affect the micronutrient (Fe, Mn, Zn, and Cu) concentrations when compared with the control (Figure 4). The shoot and root Fe concentrations were significantly reduced with the highest Cd levels. While the maximum reduction in the shoot was found with 200 µM Cd applications (25.9%), it was found with 100 µM Cd applications (42.9%) in the root

(Figure 4A). The shoot Mn concentrations showed a tendency to decrease in all Cd levels. However, all Cd levels (25, 50, 100, 150, and 200 µM) caused a dramatic decrease in root Mn concentration by 61.6%, 50.0%, 48.2%, 53.7%, and 65.3%, respectively (Figure 4B).

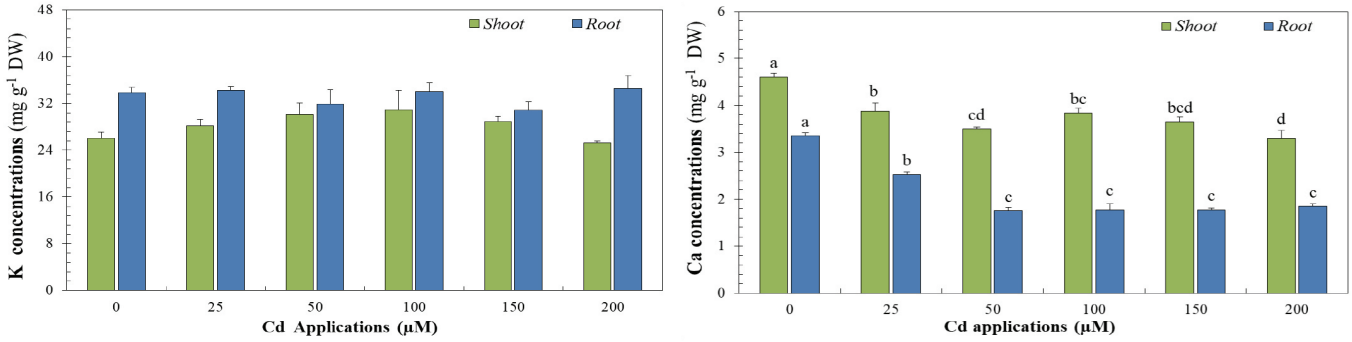

Figure 3- The effects of Cd toxicity on K and Ca concentrations in shoot and root in lavandin (Bars indicate means of three replicates ± SE. Different letters on the bars for each parameter differ significantly according to DMRT)

Likewise, increasing Cd levels caused a notable decrease in shoot and root Zn concentrations. In the shoots, these decreases at 25, 50, 100, 150, and 200 µM were 5.3%, 19.7%, 29.0%, 22.4%, and 46.6%, respectively. In the roots, similarly, the decreases were 63.3%, 67.6%, 77.5%, 79.1%, and 79.4%, respectively (Figure 4C). However, only the 100 and 200 µM Cd levels caused notable increases in the shoot Cu concentration by 38.7% and 11.1%, respectively. All Cd levels increased the root Cu concentrations and these increases were found to be over 2-fold in comparison with the control (Figure 4D).

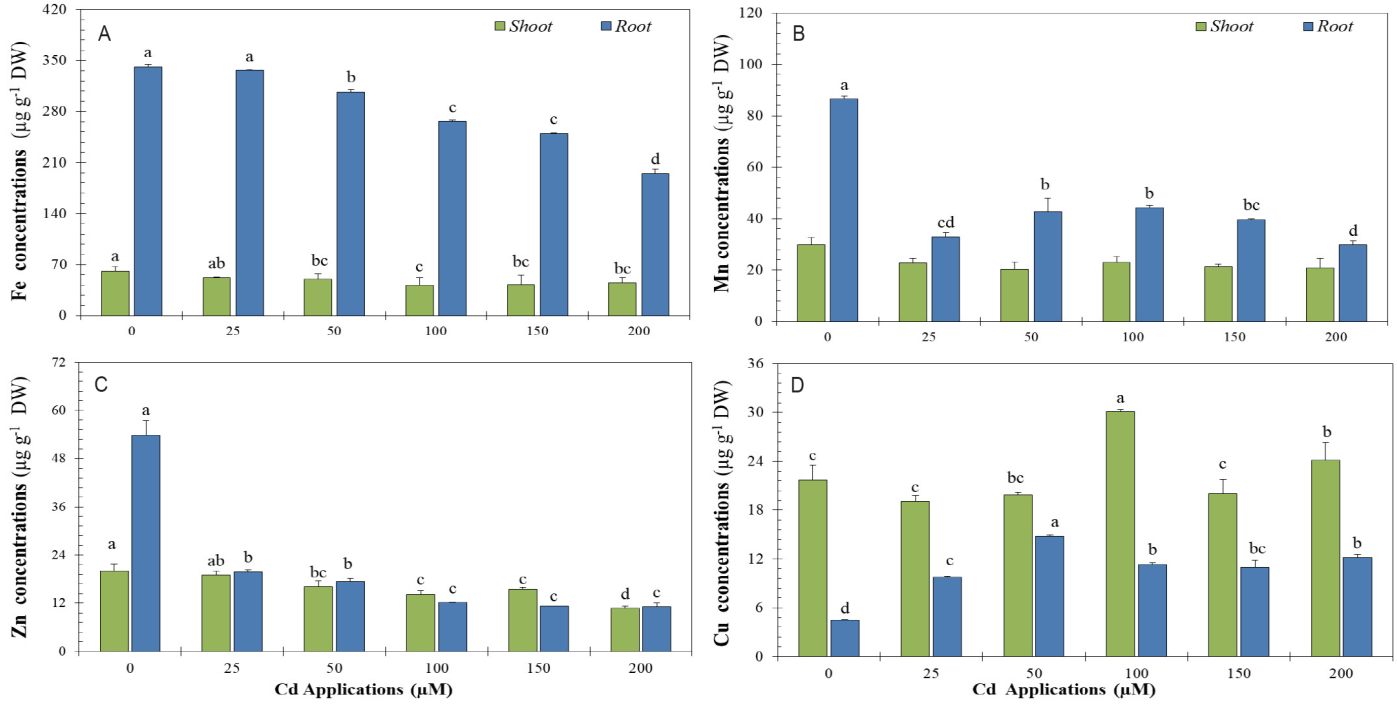

Figure 4- The effects of Cd toxicity on micronutrient concentrations in shoot and root in lavandin (Bars indicate means of three replicates ± SE. Different letters on the bars for each parameter differ significantly according to DMRT)

The BCF of Fe in lavandin (shoots and roots) reduced significantly with all Cd levels, compared to the control (Table 2). Higher Cd levels (50, 100, 150, and 200 µM) decreased the shoot BCF of Fe by 17.6%, 30.7%, 30.3%, 25.8%, respectively. These decreases were by 10.2%, 21.9%, 27.0%, and 43.1%, respectively, in the root BCF of Fe. Similar decreases in the BCF of Zn were found in both shoot and root. While 25, 50, and 150 µM Cd levels caused a decrease in shoot BCF of Cu, 100 and 200 µM Cd levels increased this parameter by 38.6% and 11.0%, respectively. All Cd levels increased the root BCF of Cu when compared to the control. The maximum

increase in root BCF of Cu was achieved with 50 µM Cd levels (Table 2). With all Cd levels (25, 50, 100, 150, and 200 µM), the BCF of Mn in roots significantly decreased at 62.0%, 50.6%, 48.8%, 54.2%, and 65.7%, respectively, compared to the control.

Table 2-The effect of Cd toxicity on bio-concentration of heavy metal ions in lavandin

Treated Cd (µM)	*BCF of Fe*		*BCF of Mn*		*BCF of Zn*		*BCF of Cu*	
	Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
0	24.4±1.24^{a}	137±2.69^{a}	59.3±0.50	173.0±2.45^{a}	401±34.3^{a}	1078±71.5^{a}	1084±92.2^{bc}	226±3.21^{d}
25	20.9±0.65^{ab}	135±0.55^{a}	45.6±3.50	5.7±3.13^{cd}	380±21.4^{ab}	396±9.47^{b}	957±36.8^{c}	488±5.70^{c}
50	20.1±1.28^{bc}	123±2.87^{b}	40.2±3.61	85.4±10.1^{b}	322±28.3^{bc}	349±12.7^{b}	996±12.4^{bc}	740±4.52^{a}
100	16.9±0.89^{c}	107±4.01^{c}	45.9±2.91	88.6±1.65^{b}	285±18.5^{c}	242±3.92^{c}	1502±15.7^{a}	568±24.2^{b}
150	17.0±0.32^{bc}	100±5.12^{c}	42.9±8.31	9.2±0.87^{bc}	311±6.89^{c}	224±1.56^{c}	99±89.1^{bc}	550±41.9^{bc}
200	18.1±2.31^{bc}	78±3.03^{d}	41.8±1.71	59.4±3.48^{d}	214±11.5^{d}	222±18.8^{c}	1203±109^{b}	610±18.5^{b}
F-test	**	***	ns	***	***	***	**	***

All values are the average of three replicates (means ± SE, n= 3). Different letters in the same column differ significantly according to the DMRT. *F*-test shows significant difference at $^{***}p<0.001$; $^{**}p<0.01$; ns: not significant

Increasing the Cd levels significantly affected the TF of metallic ions in comparison to the control, except for TF of K (Table 3). The TF level of K and Ca showed an increasing tendency with all Cd levels, with the exception of TF of K at the 200 µM Cd level. A significant difference in TF of Fe with Cd levels was not found, but a 200 µM Cd level provided a 30.3% increase. All Cd levels (25, 50, 100, 150, and 200 µM) caused notable increases in TF of Mn by 2.6-, 2.5-, 3.4-, 3.5-, and 2.7-fold and in TF of Cu by 2.1-, 1.4- 1.5-, 1.6-, and 2,1-fold, respectively. However, the same Cd levels caused notable decreases in TF of Zn by 59.1%, 71.8%, 44.5%, 61.6%, and 58.9%, respectively.

Table 3- The effect of Cd toxicity on translocation (TF) of metallic ions in lavandin

Treated Cd (µM)	*TF of K*	*TF of Ca*	*TF of Fe*	*TF of Mn*	*TF of Zn*	*TF of Cu*
0	0.78±0.07	1.45±0.12^{b}	0.178±0.01^{b}	0.37±0.02^{c}	4.79±0.39^{a}	0.34±0.01^{c}
25	0.82±0.04	1.75±0.21b	0.155±0.01^{b}	0.96±0.07^{b}	1.96±0.10^{c}	0.70±0.05^{a}
50	0.95±0.03	1.93±0.14^{b}	0.164±0.01^{b}	0.92±0.07^{b}	1.35±0.02^{c}	0.48±0.05^{bc}
100	0.91±0.06	2.72±0.39^{b}	0.159±0.00^{b}	1.27±0.10^{a}	2.66±0.13^{b}	0.52±0.03^{b}
150	0.95±0.10	2.07±0.02^{ab}	0.171±0.01^{b}	1.29±0.03^{a}	1.84±0.25^{c}	0.54±0.10^{ab}
200	0.71±0.07	1.84±0.28^{b}	0.232±0.03^{a}	0.99±0.13^{b}	1.97±0.12^{c}	0.71±0.02^{a}
F-test	ns	*	*	***	***	**

All values are the average of three replicates (means ± SE, n= 3). Different letters in the same column differ significantly according to the DMRT. *F*-test shows significant difference at $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$; ns: not significant

A significant interaction was found between increasing Cd levels and net accumulation of Cd, Fe, and Zn was found as compared to the control (Table 4). All Cd levels significantly increased the NA of Cd, with the highest increase 97-fold at 150 µM Cd level. However, increasing Cd levels decreased the NA of Fe and Zn. These decreases in NA of Fe were significant only at the 150 and 200 µM Cd levels by 36.0% and 46.1%, respectively. Likewise, the NA of Zn considerably decreased with 50, 100, 150, and 200 µM by 36.6%, 54.2%, 31.7%, and 61.3%, respectively. A non-significant interaction between increasing Cd levels and NA of K and Ca was found in lavandin (Table 4).

Table 4- The effect of Cd toxicity on the net accumulation (NA) of metallic ions via roots in lavandin

Treated Cd (µM)	*NA of Cd*	*NA of Fe*	*NA of Mn*	*NA of Zn*	*NA of Cu*	*NA of K*	*NA of Ca*
	(µg g^{-1} DW)					*(mg g^{-1} DW)*	
0	25.1±1.80^{d}	841.9±23.1^{a}	332.4±22.8	217.6±7.37^{a}	181.3±3.40	249.2±18.0	41.58±4.38
25	864±10.8^{cd}	806.6±24.0^{a}	238.0±9.35	192.2±9.83^{ab}	182.6±8.79	290.8±25.4	37.26±0.77
50	1316±41.6^{bc}	679.6±36.5^{ab}	193.5±24.0	138.0±19.6^{cd}	163.4±16.5	254.0±10.3	27.10±3.73
100	1821±157^{ab}	667.9±71.0^{ab}	188.0±24.9	99.6±12.2^{de}	201.9±35.0	224.6±25.4	25. 78±4.36
150	2414±597^{a}	538.7±111^{bc}	243.5±76.3	148.6±19.0^{bc}	186.8±32.5	287.4±49.1	33.97±5.88
200	1729±411^{abc}	453.8±83.5^{c}	172.0±44.0	84.3±21.5^{e}	179.5±50.4	202.8±53.7	24.08±5.34
F-test	**	*	ns	***	ns	ns	ns

All values are the average of three replicates (means ± SE, n=3). Different letters in the same column differ significantly according to the DMRT. *F*-test shows significant difference at $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$; ns: not significant

4. Discussion

Cd, which has no known physiological function in plants, has serious toxic effects on plants (Wang et al. 2007). Depending on the species or genotype, plants may show toxicity symptoms in the presence of more than 5-10 mg kg^{-1} of Cd in the rooting media (Mengel & Kirkby 2012). Typical toxicity symptoms of Cd include chlorosis and stunted growth (Jali et al. 2016). Also, toxic Cd levels inhibit plant growth, photosynthetic activity (Ekmekçi et al. 2008), adversely affect nutrient uptake and balance, and may accumulate in plant organs (Goswami & Das 2015). In our study, increasing the Cd levels caused a significant decrease in shoot and root FW and DW (Figure 1,5) and Chl *a*, *b*, *a+b,* and Car contents (Figure 2A). These decreases may be associated with the phytotoxic effect of Cd on the synthesis of the cell wall (Parrotta et al. 2015) and enzyme activities, and photosynthetic electron transport (Hassan et al. 2005). Excess Cd also reduces Fe and Zn uptake (Figure 4A, 4C), resulting in leaf chlorosis (Haider et al. 2021). However, higher Cd levels can inhibit chlorophyll (Chl) biosynthesis and reduce the activity of enzymes involved in CO_2 fixation and thus cause a disruption in chloroplast biosynthesis (Raziuddin et al. 2011). Sandalio et al. (2001) found that Cd affected both the leaves and roots in pea plants and significantly inhibited transpiration and photosynthesis rate, as well as cause a general nutrient imbalance in the plant. In addition, Cd may lead to the impairment of photosynthetic machinery (Irshad et al. 2021) and it may displace with Mg which are central atoms of the Chl molecule (Gill 2014). On the other hand, excess Cd caused an increase in the MP value of lavandin leaves (Figure 2B). These increases could be explained by the direct effects of excess Cd on proteins and lipids, which are the main components of membranes, or by inducing lipid peroxidation (Fodor et al. 1995). Ekmekçi et al. (2008) found that Cd applications might result in membrane damage and deterioration of membrane integrity in maize leaves. Barceló & Poschenrieder (1990) stated that metal toxicity causes a reduction in water content and thus affects the plasma MP and, particularly, Cd interacts with the water balance.

Figure 5- The toxic effect of cadmium on growth of lavandin

Depending on the increasing Cd levels, shoot and root Cd concentrations also increased (Table 1). Cd concentration in roots is generally higher than in shoots (Ekmekçi et al. 2008; Ehsan et al. 2015). In this study, it was found in the roots to be over 120-fold than in the shoots. This indicated that the plants accumulated Cd in their roots and did not carry it to the aerial organs due to some barriers (Pinto et al. 2004). Di Toppi & Gabbrielli (1999) explained that the reduction in the transfer of Cd from root to shoot could be due to metal immobilization in the cell wall. Angelova (2012) reported that some medicinal and aromatic plants accumulate a large portion of heavy metals in their roots and transfer very little of these heavy metals to the upper organs, and have a high growth potential in heavy metal-contaminated areas.

The Cd uptake by plant roots occurs through the same transmembrane carriers used to uptake Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+} (Papoyan & Kochian 2004) and Cd ion could compete with these cations to access the cell of plants through the transport systems (Barceló & Poschenrieder 1990). Thus, Cd could interfere with the uptake and transport of Ca, P, Mg, K, and Mn (Nazar et al. 2012). Decreases in Ca concentration (Figure 3) and Fe, Mn, and Zn concentrations (Figure 4) in shoots and roots could be attributed to the interactions of these nutrients with Cd. This effect could be ascribed to dysfunctions of the membrane integrity caused by the displacement of Ca in the cell wall with Cd. In line with this, Nada et al. (2007) reported that the roots and leaves of almond seedlings exposed to 100 and 150 µM Cd caused a decrease in Ca, Mg, and K.

The BCF indicates the ability of plants to absorb a heavy metal from the rooting medium, while TF indicates the ability of heavy metals to transfer from the root medium to the upper organs such as stem, leaf, flower, and fruit. Both concepts are typically used to determine the phytoremediation capacity of plants (Ghosh & Singh 2005) and tend to decrease depending on increasing concentrations of heavy metals in rooting media (Zhao et al. 2004). The results from this study reveal that metallic ion accumulation was reduced in

the shoots and roots with increasing Cd levels. The fact that the BCF of Cd in the roots was greater than the BCF of Cd in the shoot indicates that the root accumulates more Cd than in the shoot (Table 1). It is known that many plants reduce the uptake of metals to their aerial parts and preferentially accumulate and store them in their roots (Usman et al. 2019) or bind them to amino acids, proteins, and peptides (Pál et al. 2006). Likewise, the BCF of Fe, Zn, and Mn was reduced by increasing Cd levels (Table 2). The higher metallic ion concentrations in the roots compared to the shoots (Figure 4) can be explained by the inability to transport them from the roots to the shoots. Reductions in translocation from root to the shoot appear to be a common feature in plants exposed to heavy metals. The relatively high accumulation of Cd in plant roots could partially be due to the binding of the Cd ions to some specific sites in the root cell wall (Zhu et al. 1999). Chen et al. (2021) reported that heavy metal accumulation in rice and maize plants followed the order root > stem = leaf > cereal.

The net accumulation of metallic cations via roots indicates the amount of these cations deposited by a unit root in the upper organs of a plant (Moradi & Ehsanzadeh 2015). While the increase in net accumulation of Cd is likely to depend on the accumulation of Cd ions in rooting media, a decrease in net accumulation of Fe and Zn depends on excessive Cd in rooting media and its toxic effects (Table 4). It also could be related to the antagonistic effects of Cd ions on Fe and Zn ions that have been in the same redox-active group (Singh et al. 2016). Our results are in line with those reported by Moradi & Ehsanzadeh (2015) who studies Cd ions in safflower plants.

In our study increasing Cd levels caused an increase in the TAR of Cd (Table 1). This increase in the TAR of Cd could be attributed to the excess Cd^{2+} concentration present in the rooting media. Campbell (1995) stated that the metal accumulation is controlled by the free ion concentration. Likewise, Kösesakal et al. (2011) reported that higher metal presence in rooting media causes an increase in metal uptake and significantly increases TAR value.

5. Conclusion

This study concludes that lavandin accumulates increasing amounts of Cd in the tissues at increasing Cd levels in the rooting medium, with negative effects on growth and biomass production. The roots are the main metal sinks due to a low translocation from roots to the shoot, suggesting a defence or tolerance mechanism to avoid toxic levels in physiologically most active apical tissues. However, Cd could interact with metal ions, especially divalent cations (Ca, Fe, Zn, and Mn), and could prevent these ions from fulfilling their role in mineral nutrition by reducing their uptake by roots. Meanwhile, a significant correlation has been found between the net accumulation of Cd, Fe, and Zn via roots of lavandin and Cd concentrations in the rooting medium.

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